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INSTITUTE REPORT NO. 191

ACUTE ORAL TOXICITY (LD_{50}) OF 4--NITROPHENYL MONOCHLOROMETHYL (PHENYL) PHOSPHINATE (TA009) IN MALE RATS

CRAIG W. WHITE, DVM, CPT VC
JUSTO RODRIGUEZ, BS, SP4
and
THOMAS P. KELLNER, BS, SP5

TOXICOLOGY GROUP
DIVISION OF RESEARCH SUPPORT

OCTOBER 1984 Toxicology Series 55

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

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Acute Oral Toxicity (LD_{50}) of 4-Nitrophenyl Monochloromethyl (Phenyl) Phosphinate (TA009) in Male Rats (Toxicology Series 55)--White et al

In conducting the research described in this report, the investigation adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care, Institute of Laboratory Animal Resources, National Research Council.

This material has been reviewed by Letterman Army Institute of Research and there is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. *(AR 360-5)*

Edwin S Beatrice 4 Oct 84
(Signature and date)

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REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER LAIR Institute Report No. 191	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Acute Oral Toxicity (LD_{50}) of 4-Nitrophenyl Monochloromethyl (Phenyl) Phosphinate (TA009) in Male Rats		5. TYPE OF REPORT & PERIOD COVERED Final 22 Sep - 19 Oct 82
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Craig W. White, DVM, CPT VC Justo Rodriguez, SP4, BS Thomas P. Kellner, SP5, BS		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS Toxicology Group, Division of Research Support, Letterman Army Institute of Research Presidio of San Francisco, CA 94129-6800		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 35162771A875
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Command Fort Detrick Frederick, MD 21701-5012		12. REPORT DATE October 1984
		13. NUMBER OF PAGES 28
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (of this report) UNCLASSIFIED
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

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17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Toxicology, Organophosphinate, Acetylcholinesterase Inhibitor, 4-Nitrophenyl Monochloromethyl (Phenyl) Phosphinate

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The acute oral toxicity of 4-nitrophenyl monochloromethyl (phenyl) phosphinate was determined in male, albino, Sprague-Dawley rats by using the oral gavage dose method. LD_1, LD_{50}, and LD_{95} with the 95% confidence limit were calculated by probit analysis. The LD_{50} was 203 mg/kg with the 95% confidence limit (142 mg/kg, 292 mg/kg). The formulation falls in the very toxic range.

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ABSTRACT

The acute oral toxicity of 4-nitrophenyl monochloromethyl (phenyl) phosphinate was determined in male, albino, Sprague-Dawley rats by using the oral gavage dose method. LD_1, LD_{50}, and LD_{95} with the 95% confidence limit were calculated by probit analysis. The LD_{50} was 203 mg/kg with the 95% confidence limit (142 mg/kg, 292 mg/kg). The formulation falls in the very toxic range.

Key Words: Toxicology, Organophosphinate, Acetylcholinesterase Inhibitor, 4-Nitrophenyl Monochloromethyl (Phenyl) Phosphinate

PREFACE

TYPE REPORT: Acute Oral Toxicity (LD_{50}) GLP Study Report

TESTING FACILITY: U.S. Army Medical Research and Development Command
Letterman Army Institute of Research
Division of Research Support
Presidio of San Francisco, CA 94129-6800

SPONSOR: U.S. Army Medical Research and Development Command
U.S. Army Institute of Medical Research and Chemical Defense
Aberdeen Proving Ground, MD 21010

PROJECT: 35162772A875 Medical Defense Against Chemical Agents
WU 304 Toxicity Testing of Phosphinate Compounds
APC TL04

GLP STUDY NUMBER: 82029

STUDY DIRECTOR: COL John T. Fruin, DVM, PhD, VC
Diplomate, American College of
Veterinary Preventive Medicine

PRINCIPAL INVESTIGATOR: CPT Craig W. White, DVM, VC

CO-PRINCIPAL INVESTIGATOR: SP4 Justo Rodriguez, BS

PATHOLOGIST: LTC Paul W. Mellick, DVM, PhD, VC
Diplomate, American College of
Veterinary Pathologists.

STATISTICIAN: Virginia L. Gildengorin, PhD

DATA MANAGER: Carolyn M. Lewis, MS

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: 4-Nitrophenyl Monochloromethyl (Phenyl) Phosphinate,
LAIR Code TA009

INCLUSIVE STUDY DATES: 22 September-19 October 1982

OBJECTIVE: The objective of this study was to determine the acute oral toxicity of (LD_{50}) 4-nitrophenyl monochloromethyl (phenyl) phosphinate in male Sprague-Dawley rats.

ACKNOWLEDGMENTS

The authors wish to thank SP5 Leonard J. Sauers, MS, and SP5 Evelyn M. Zimmerman for assistance in performing this research. A special debt of gratitude is due Claire N. Lieske, US Army Research Institute of Chemical Defense, who provided test compound, continued advice, and willing inter-agency support.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY

We, the undersigned, believe the study number 82029 described in this report to be scientifically the sound and the results in this report and interpretations to be valid. The study was conducted to comply, to the best of our ability, with the Good Laboratory Practice Regulations outline by the Food and Drug Administration.

21 Dec 83
JOHN T. FRUIN / DATE
COL, VC
Study Director

8 Mar 84
JUSTO RODRIGUEZ, BS / DATE
SP5, USA
Co-Principal Investigator

PAUL W. MELLICK, PhD / DATE
LTC, VC
Pathologist

8 Mar 84
THOMAS P. KELLNER, BA/ DATE
SP5, USA
Co-Principal Investigator

CRAIG W. WHITE / DATE
CPT, VC
Principal Investigator

VIRGINIA D. GILDENGORIN, PHD / DATE
Statistician

8 Mar 84
CAROLYN M. LEWIS, MS / DATE
Data Manager

DEPARTMENT OF THE ARMY
LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

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ATTENTION OF:

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3 Jul 84

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SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 82029 the following inspections were made:

01 Oct 82

04 Oct 82

12 Oct 82

19 Oct 82

The report and raw data for this study were audited on 26 Jun 84.

Routine inspections with no adverse finding are reported quarterly, thus these inspections are also included in the 4 Jan 83 report to Management and the Study Director.

Nelson R Powers

NELSON R. POWERS, Ph.D.
DAC
Chief, Quality Assurance Unit

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Acute Oral Toxicity (LD_{50}) of 4-Nitrophenyl Monochloromethyl (Phenyl) Phosphinate (TA009) in Male Rats (Toxicology Series 55)--White et al

One mission of the US Army Medical Research and Development Command is to develop compounds for prophylaxis agair organophosphate intoxication. The organophosphinat .ass of chemical compounds are promising candidates in this effort. . is hoped that a compound can be found with relatively minor side-e .cts at doses required to provide significant systemic protection. phosphinates represent a strategy of prophylaxis whereby a critical percentage of the available acetylcholinesterase is protected from chemical agent by binding with a compound, such as 4-nitrophenyl monochloromethyl (phenyl) phosphinate, from which the enzyme may be reactivated by standard antidotal therapy (1-4).

Objective of the Study

The objective of this study was to determine the acute oral toxicity (LD_{50}) of 4-nitrophenyl monochloromethyl (phenyl) phosphinate in male Sprague-Dawley rats.

MATERIALS

Test Substance

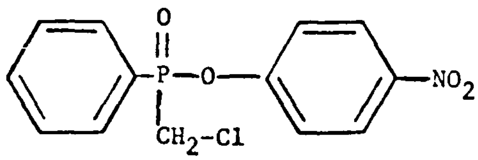
Chemical name: 4-Nitrophenyl Monochloromethyl (Phenyl) Phosphinate

LAIR Code: TA009

Code Name: MCP, CMP

Chemical Abstract Service Registry Number: None known.

Chemical structure:



Empirical formula: $C_{13}H_{11}ClNO_4P$

The test compound was received from the US Army Medical Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010 on 23 June 1982. The test chemical was stored at refrigeration temperature (as suggested by the sponsor) until time of compounding with the vehicle just before dosing. Detailed chemical data on the test compound are given in Appendix A.

Vehicle

Since phosphinates hydrolyze readily in aqueous solutions, a vehicle which would minimize the rate of hydrolysis was required. A mixture of Tween 80™ (Fisher Scientific Company, Fairlawn, NJ), ethanol, and citrate buffer (pH 3.2) was chosen. Additional information on the vehicle composition is given in Appendix A.

Animals

Fifty male, albino, Sprague-Dawley rats from Charles River Breeding Laboratories, Inc., Kingston, NJ, were studied. Ear tags were used to identify each animal individually. Tag numbers from 82D00522 to 82D00582 (with exclusions) were used. The rats' weights on 23 Sep 82 ranged from 123 to 158 g. Additional animal data are given in Appendix B.

Husbandry

The animals were housed individually in stainless steel mesh drawer rack cages. No bedding was used in any of the cages.

Diet consisted of Certified Purina Rodent Chow #5002 (Ralston Purina, Checkerboard Square, St. Louis, MO) ad lib. Water was provided with automatic Lixit dispensers.

The temperature maintained throughout this study was 26 ± 2°C with a relative humidity of 40 ± 5%. The photoperiod was 15 hours of light daily (0500 - 2000 hours).

METHODS

Group Assignment/Acclimation

The Beckman TOXSYS Animal Allocation Program was used to assign seven males to each of seven study groups. This program incorporates a weight-biased stratification procedure for allocating the aniamls to the various study groups.

The animals were acclimated for 14 days before dosing. During the acclimation period the animals were observed daily for signs of illness.

Dose Levels

Since the Approximate Lethal Dose (ALD) study indicated that the LD_{50} would be between 200 and 300 mg/kg, doses of 150 mg/kg, 200 mg/kg, 250 mg/kg, 300 mg/kg, and 400 mg/kg were selected for the LD_{50} determination. The amount of dosing solution each animal received was based upon the animal's weight, the desired dose level, and the compound concentration in solution. The dose level was increased volumetrically rather than by varying concentration. The volume administered ranged from 0.92 ml to 2.7 ml. The cage control group was untreated. The vehicle group received 2.0 ml of the vehicle. The dosing was by oral gavage.

All animals were dosed between 0920 and 1055 hours, on 4 October 1982. Sterile, disposable syringes (Becton, Dickinson & Co., Rutherford, NJ) fitted with 16-gauge, 3-inch, ball-tipped feeding tubes (Popper & Sons, Inc., New Hyde Park, NY) were utilized. The dosing procedures were conducted without animal sedation or anesthesia.

Compound Preparation

A 3.0 percent phosphinate solution was prepared as described in Appendix A. Results of hydrolysis measurements of the dosing solution performed immediately after preparation and within 30 minutes after administration are given in Appendix A.

Test Procedures

The animals were observed for signs of acute toxicity and for mortality during the dosing procedure. Also, they were observed at 1200 and 1615 hours. Observations were conducted daily for the remainder of the study. Body weights were recorded just before dosing and twice weekly until death or study completion. Appendix C contains a complete listing of observation periods.

All animals assigned to this study were subjected to a complete gross necropsy. Animals which survived the entire study period underwent necropsy immediately after sacrifice by barbituate overdose.

Statistical analyses were performed on the study data. The LD_1, LD_{50}, and LD_{95} were derived by Bliss probit analysis, as described by Finney (5). The program, PROBIT, written for the Data General Model C330 Computer, was used to determine the probit curve and lethal dose values. The statistician's report appears in Appendix D.

The dosing phase of this study was accomplished according to the protocol and applicable amendments, except that the volumes administered to Group 5 were divided into two equal doses. Double dosing of this group was required in an attempt to prevent reflux of the calculated dose. The second dose was delivered within one hour of the first.

Raw Data and Final Report Storage

A copy of the final report, study protocols, raw data, retired SOPs and aliquot of the test compound will be retained in the LAIR Archives.

Deviations from Protocol

A total of four dose groups were used in the lethal dose calculations. The original protocol stated that five groups would be used.

RESULTS

Mortality

Table 1 lists the compound-related deaths by group and the percent mortality.

Lethal Dose Calculations

Lethal dose (LD) values were calculated by probit analysis as described by Finney (5). Data from dose group 5 was not included in the probit analysis determination since the dose these animals actually received was considerably lower than that administered due to problems with the double-dose method used. The computer-generated lethal dose values for selected percentages of the population are presented in Table 2.

Table 1

Compound-Related Deaths by Group

Group	Dose Level mg/kg	Compound-Related Death/ Number in Group	Percent Mortality
1	150	3/7	43
2	200	2/7	29
3	250	5/7	71
4	300	5/7	71
5	400	5/7*	71*
6	Vehicle Control	0/7	0
7	Untreated Controls	0/7	0

*Group eliminated from the probit analysis because of uncertainty of dose levels administered. The double-dose method used in this group was responsible for the uncertainty. The first dose produced severe irritation in the G.I. tract (Pathology Report, Appendix E). Severe retching and salivation were observed after the second dose was administered, leading to the conclusion that a large portion of the second dose was not absorbed.

Table 2*

Lethal Dose (LD) Levels in TA009 in Male Rats

Percent Population	Lethal Dose (mg/kg)	95% Confidence Limit (mg/kg)
LD_{1}	37.7	(3.5, 406)
LD_{50}	203	(142, 292)
LD_{95}	668	(149, 2991)

*Statistician Report (Appendix D)

Clinical Observations

The primary toxic signs attributable to the test compound were difficulties with equilibrium and disturbances in gait (30 or 35 animals), a humpback posture (29 of 35 animals), inactivity (31 of 35 animals), and sluggishness (20 of 35 animals). A distinct yellow discoloration of the animal coat also occurred in 26 of 35 animals. These signs increased in frequency and duration with increasing dose levels. Seven animals developed extremely foamy salivation and gave the appearance of retching.

Decreased rate and depth of respiration was observed frequently, especially in Groups 3, 4, and 5. The incidence of rough hair coat increased in a dose-response relationship. A mild depression of the Righting reflex was noted in some animals.

Gross Pathological Observations

The mortalities appear to have been caused by the test compound. The dose-response relationship demonstrated with the test compound appears to be valid. The veterinary pathologist's report is included as Appendix E.

DISCUSSION

The calculated LD_{50} for 4-nitrophenyl monochloromethyl (phenyl) phosphinate in male Sprague-Dawley rats was 203 mg/kg with a 95 percent confidence limit (142 mg/kg, 292 mg/kg). The LD_{50} is within the very toxic range (6).

Clinical signs of toxicity included depression, inactivity, ataxia, loss of equilibrium and gait, decreased respiratory rate, decreased respiratory depth, rough hair coat, humpback posture, salivation, retching with reflux of dosing material, and death.

An interesting phenomenon observed during this study was the plateau of lethality that occurred with increasing dose levels. This was attributed to the inability of animals in Dose Group 5 to retain the entire dose administered because of retching and excessive salivation. Salivation and retching are parasympathetic manifestations of the cholinesterase inhibition produced by phosphinate compounds. Although no chemical analyses were performed to confirm that the saliva and refluxed material contained 4-nitrophenyl monochloromethyl (phenyl) phosphinate, its hydrolysate, *p*-nitrophenol, is the same bright yellow color as the material observed in the saliva.

The double-dose method (used in Group 5 rats because of large dosing volumes) was not successful and led to the elimination of this group from the probit analysis. All of the animals in this group failed to absorb some portion of the second dose because of the severe stomach irritation produced by the first dose (Pathology Report, Appendix E).

CONCLUSION

The LD_{50} for 4-nitrophenyl monochloromethyl (phenyl) phosphinate (TA009) was determined to be 206 mg/kg in male Sprague-Dawley rats. The formulation is considered to be very toxic (6).

RECOMMENDATION

4-Nitrophenyl monochloromethyl (phenyl) phosphinate (TA009) should be considered for further safety testing for eventual human use, provided efficacy is verified.

REFERENCES

1. Lieske CN, Clark JH, Meyer HG, Lowe JR, Lawson MA, Lennox WJ. The concept and chemistry of organophosphinates as prophylactic agents in organophosphate intoxication. Toxicology Research Projects Directory 1981:7.

2. Lieske CN, Meyer HG, Clark JH, Lowe JR, King JW. Spontaneous reactivation of bovine erythrocyte acetylcholinesterase inhibited by five organophosphinates. Aberdeen Proving Ground, MD: U.S. Army Medical Bioengineering Research and Development Laboratory, 1979.

3. Horton GL, Lieske CN, Lowe JR. Phosphinate inhibition studies of cholinesterases. Pestic Sci 1978; 9:135-138.

4. Hodgson E, Guthrie FE, eds. Introduction to biochemical toxicity. New York: Elsevier Science Publishing Co., Inc., 1980:193-223.

5. Finney DJ. Probit analysis. 3rd ed. Cambridge: Cambridge University Press, 1971:20-80.

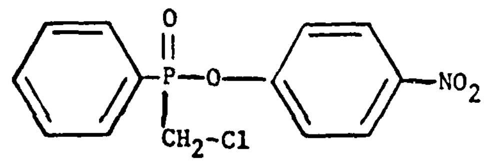
6. Doull J, Klaassen CD, Amdur MO, eds. Casarett and doull°s toxicology. 2nd ed. New York: MacMillan Publishing Co., Inc., 1980:18-22, 365-408.

APPENDICIES

CHEMICAL DATA

Chemical name: 4-Nitrophenyl Monochloromethyl (Phenyl) Phosphinate

Structural formula:



Empirical formula: $C_{13}H_{11}ClNO_4P$

pH: N/A non-aqueous

Physical state: White crystalline solid

Boiling point: N/A

Melting point: 77-78.5 C

Stability: Dr. Lieske (Biomedical Laboratory, Aberdeen Proving Ground, Aberdeen, MD 21005) indicated the compound would remain stable for two years if refrigerated.

Name of contaminants and percentages: unknown

Manufacturer: Ash Stevens
Detroit Research Park
5861 John C. Lodge Freeway
Detroit, MI 48202

Manufacturer Lot Number: MP-07-201

Dosing Solution: 4-nitrophenyl monochloromethyl (phenyl) phosphinate formulated with Tween 80, EtOH, and citrate buffer (LAIR SOP-OP-STX-45, Preparation of Compounds Unstable in Water for SLRL Assay).

A 3.0 percent phosphinate solution was prepared with 2.40 g 4-nitrophenyl monochloromethyl (phenyl) phosphinate. 16.0 ml Tween 80™, 8.0 ml (100 %) ethanol, 56.0 ml citrate buffer (50 mM) at a pH of 3.2. The vehicle was the same as above without phosphinate.

pH: 3.2

Physical state: liquid/clear yellow

Boiling point: N/A

Melting point: N/A

Compound refractory: N/A

Contaminants (percentages): Not available

Analysis of Dosing Solution for Hydrolysis:

The phosphinate solution and vehicle were assayed for intact and hydrolyzed phosphinate immediately after preparation and dosing. P-nitrophenol, a product of phosphinate hydrolysis, was quantitated spectrophotometrically at 400 nm using a value of 18,300 for the molar extinction coefficient. Absorbance was measured with a Gilford 2400-S Spectrophotometer in accordance with LAIR SOP-OP-STX-49 "Spectophotometric Measurement of P-nitrophenol for Phosphinate Determination." The concentration of unhydrolyzed phosphinate in the dosing solution was determined from the difference in p-nitrophenol concentration before and after NaOH hydrolysis. The initial hydrolyzed phosphinate was divided by the total hydrolyzed phosphinate to obtain the percent hydrolysis for each solution. The "predosing" measurements of hydrolysis were less than 7% while the "after dosing" measurements were less than 15%. Hydrolysis of the phosphinate solution during dosing averaged 6%.

APPENDIX A (concluded)

ANIMAL DATA

Species: Rattus norvegicus (albino laboratory rat)

Source: Charles River Breeding Laboratories, Inc.
Kingston, NJ

Sex: Male

Date of Birth: 13 August 1982

Method of randomization: Weight bias, stratified animal allocation (Beckman TOXSYS Animal Allocation System)

Animals in each group: 7 male animals

Condition of animals at start of study: Normal

Body weight range at dosing: 165-206 g

Identification procedures: Ear tagging procedure (SOP OP-ARG-1), tag numbers between 82D00522 to 82D00582 with exclusions.

Pretest conditioning: Quarantine/acclimation 22 September - 3 October 1982

Justification: The laboratory rat has proven to be sensitive and reliable system for lethal dose determination.

HISTORICAL LISTING OF STUDY EVENTS

Date	Event
22 Sep 82	Fifty-two male Sprague-Dawley rats were received at LAIR. Rats were housed individually and were ear-tagged. Animals were weighed and 2 animals were submitted to quality control necropsy.
24 Sep 82	Animals were randomized, divided into dose groups and weighed.
4 Oct 82	Animals were weighed, dosed, and observed. Necropsy was performed on all animals.
4-18 Oct 82	All animals were observed daily for mortality and clinical signs.
22,24,27 Sep 82 1,4,8,12,15 and 19 Oct 82	All animals weighed.
19 Oct 82	All surviving animals were observed, weighed, sacrificed, and gross necropsies performed.

STATISTICAL ANALYSIS

Bliss method of probit analysis was used to determine the LD_1, LD_{50}, and LD_{95} values along with the corresponding 95% confidence limits (Table 2). The program, PROBIT, was used to determine the probit curve and the lethal dose values. The probit regression line fit to the data was:

$$Y = -2.3 + 3.2 \log x$$

A t-test was performed to test the hypothesis of a zero slope. The slope was not found to be significantly different from zero. Therefore the lethel dose values can only serve as rough estimates of lethality (as illustrated from the confidence units).

Virginia L. Gildengorin

VIRGINIA L. GILDENGORIN, PhD
DAC, Statistician
Date:

PATHOLOGY REPORT

Gross Pathology Summary and Interpretation of GLP Study 82029;
LD_{50} 4-Nitrophenyl Monochloromethyl (Phenyl) Phosphinate
(LAIR Code TA009), Male Sprague-Dawley Rats

History: The deaths of 5/7* male rats in Group 5 (400 mg/kg), 5/7 rats in Group 4 (300 mg/kg), 5/7 rats in Group 3 (250 mg/kg), 2/7 rats in Group 2 (200 mg/kg), and 3/7 rats in Group 1 (150 mg/kg) were attributed to the toxic effect of the tested compound. All deaths occurred between 73 minutes and 22 hours following administration of the test compound by gastric intubation. None of the male rats in Group 6 (vehicle control) or Group 7 (cage control) died prior to the scheduled termination of the study 14 days after administration of the test compound.

Gross changes attributable to the test compound were present in all rats that died. The most consistent lesion occurred in the gastrointestinal tract. The stomachs, small intestines and cecums of all rats that died acutely were distended with either clear or green-tinged watery material and serosal surfaces were diffusely reddened. The glandular mucosa of the stomach was also diffusely reddened in these animals. These changes were not observed in animals that survived for two weeks. They were probably due to the irritating effect of the compound with resultant excess glandular secretion, vascular congestion and possibly slight mucosal hemorrhage.

Some of the rats that died had clear or yellow fluid around the muzzle, nostrils, and/or anus. These changes occurred in 5/7 rats in group 5, 1/7 in group 4, and 3/7 in group 3. This change may have been due to reflux of material administered by gastric intubation or possibly by passage of material and/or its by-products in the feces.

Some of the rats in all treatment groups and both control groups had serous atrophy of retroperitoneal fat and slight to moderate excess of clear fluid in the peritoneal cavity. The incidence of this change by dosage group was: 3/7 in Group 1, 4/7 in Group 2, 2/7 in Group 3, 2/7 in Group 4, 2/7 in Group 5, 6/7 in Group 6, and 7/7 in Group 7. The change affected almost all animals that survived the two-week observation period and were killed at the scheduled termination of the study. Many of these animals were thin and unthrifty in appearance. No other lesions were detected nor was there evidence of concurrent disease. The cause of this change is unknown. It is not associated with administration of the test compound since animals in both cage control and vehicle control groups were affected.

Other lesions observed included dilated renal pelvis, bilateral in 1/7 rats in Group 2, mottled or tan colored kidneys in 1/7 rats in Group 3, 1/7 in

*Number of rats affected/Number of rats in group

Group 4, and 1/7 in Group 7. These changes were considered to be incidental findings unrelated to administration of the test compound.

In summary, the gross pathologic effects, in addition to death that most likely were due to single dose gastric intubation with 4-Nitrophenyl Monochloromethyl (Phenyl) Phosphinate that were observed in male Sprague-Dawley rats in this study were:

Excess glandular secretion, vascular congestion, and possible hemorrhage in the stomach, small intestine, and cecum.

Necropsies revealed no test compound-related lesions in male Sprague-Dawley rats that were killed at the termination of the study.

Paul W. Hellick

PAUL W. HELLICK, DVM, PhD
Diplomate, A.C.V.P.
LTC, VC
Chief, Pathology Services Group
Division of Research Support

3 March 1983

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ATTN: HSHA-CDM
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Institute and Research Support
Uniformed Services University
of Health Sciences
6917 Arlington Road
Bethesda MD 20014

Commander
US Army Environmental Hygiene Agency
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Washington DC 20332

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